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Determination of Phencyclidine by Radioimmunoassay

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ABSTRACT: A radioimmunoassay (RIA) was developed for measurement of phencyclidine (PCP) and its monohydroxy metabolites in urine. Anti-PCP serum was generated in rabbits and the labeled antigen was prepared by radio-iodination (^{125}I) by the Hunter-Greenwood procedure. Adjustment of the antiserum and ^{125}I-antigen concentrations resulted in a dynamic response from 0 to 200 ng/ml and as little as 2 ng/ml was detected. Reagents were stable for at least three months at 2 to 8°C. Most (95%) urine specimens from presumed nonusers contained less than 5 ng/ml and urine specimens from suspected abusers usually exceeded 100 ng/ml. Comparison of RIA with other methods for detection of PCP showed good agreement. Cross-reactivity with other commonly prescribed drugs was not encountered. The recovery of PCP was 93 to 109% over the 20 to 175 ng/ml concentration range and the reproducibility (average coefficient of variation) was ±13%. Development of the RIA for PCP has resulted in a rapid procedure that can be adapted to automated processing and is also suitable for small-scale testing.

KEY WORDS: toxicology, phencyclidine, radioimmunoassay

Phencyclidine [1-(1-phenylcyclohexyl)piperidine, PCP] is an anesthetic that is useful in veterinary applications [*1-4*]. The illicit use of the drug in humans produces clinical symptoms ranging from confusion, disorientation, stupor, and coma to death in cases of overdose [*2-8*]. The ingestion of 1 mg or inhalation of 1 to 3 mg can also produce physiological changes [*4*]. The plasma half-life of phencyclidine in humans has been reported to be 11 h [*2*]. However, a confused state may continue for as long as 15 days and psychosis may persist for four to five weeks following use [*4,5,6,9*]. Blood (plasma and serum) levels of 100 ng/ml are usually associated with coma [*10,11*].

Because of its lipophilic nature, PCP may be detected in central nervous system fluid and tissue when it is no longer found in blood or is found in trace amounts in urine [*12*]. It has been identified in urine at levels as low as 0.010 μg/ml and greater than 23 μg/ml [*4,7,13*]. The metabolites of phencyclidine in urine have been identified as an *N*-dealkylated metabolite, 1-phenylcyclohexylamine, and two hydroxylated metabolites, 1-(1-phenylcylohexyl)4-hydroxypiperidine and probably 1-(3-hydroxy-1-phenylcyclohexyl)piperidine [*14*].

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The analytical methods available for the detection and measurement of PCP include, in the order of increasing sensitivity, thin-layer chromatotraphy (TLC) [*15*], gas chromatography (GC) [*16*], and gas chromatography-mass spectrometry (GC/MS) [*17*]. These techniques presently require extraction of the biological fluid before the determination. This is time-consuming and may cause loss of PCP.

A radioimmunoassay for aminophencyclidine in dog serum was reported [*18*] using anti-aminophencyclidine rabbit serum with ^{3}H-aminophencyclidine or ^{3}H-phencyclidine as the labeled antigen.

In order to determine PCP in human urine with maximum sensitivity and specificity without the need for sample pretreatment, a radioimmunoassay has been developed with anti-PCP rabbit serum and ^{125}I-PCP antigen. This report describes the application of the radioimmunoassay for determination of PCP in human urine. In addition, limited preliminary data are presented for detection of PCP in human serum.

Materials and Methods

Reagents

Anti-phencyclidine rabbit serum was produced by injecting New Zealand White rabbits subcutaneously with immunogen at continuous weekly (1 to 8) and then monthly intervals. The immunogen consisted of 1 ml (containing 5.6 μg PCP equivalents per 5 mg of bovine serum albumin) of PCP-bovine serum albumin conjugate, prepared as an emulsion (1:1) in Freund's complete or incomplete adjuvant. Initial weekly and subsequent monthly bleedings were obtained by venipuncture and the serum was stored at −20 to 5°C until further use. Phencyclidine antiserum reagent was prepared by diluting (to bind at least 70% of the radiolabeled PCP) the anti-PCP rabbit serum in 0.05*M* phosphate-buffered saline containing 25 to 50% normal rabbit serum and 0.1% sodium azide.

Labeled PCP was obtained by radio-iodination (sodium ^{125}I, Amersham-Searle) of a PCP derivative by the Hunter-Greenwood procedure [*19*]. The antigen reagent was prepared by dilution of the chromatographically purified (Bio-Gel P-2, Bio-Rad Laboratories, Richmond, Calif.) iodinated PCP in 0.05*M* phosphate buffered saline, pH 7.2 to 7.4, containing 0.1% sodium azide, to a final radioactive concentration of 4.1×10^5 disintegrations per minute (dpm) per millilitre.

Phosphate-buffered saline, 0.05*M*, pH 7.2, was prepared by dissolving 6.9 g of monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$) and 13.4 g of dibasic sodium phosphate ($Na_2HPO_4 \cdot H_2O$) in distilled water containing 0.9% sodium chloride and 0.1% sodium azide and then adjusting the pH to 7.2 ± 0.1 and diluting to one litre.

Saturated ammonium sulfate was prepared in deionized water without neutralization.

Normal rabbit serum containing 0.1% sodium azide (Pel Freeze Biologicals, Rogers, Ark. or Gibco, Grand Island, N.Y.) was sterile-filtered (0.45 μm, Millipore Filter Corp., Bedford, Mass.).

Negative urine controls were prepared by sterile-filtering urine collected from adults. After being tested by radioimmunoassay to confirm the absence of PCP, the urine specimens were pooled and the pool was stored at 2 to 8°C for further use.

Positive urine controls were prepared by appropriate dilution of a PCP stock solution (1 mg/ml in distilled water) in pooled negative human urine or commercially obtained human serum for the serum controls.

Procedures

The radioimmunoassay was performed as follows: 0.1 ml of sample or control was added to each of a series of glass tubes (10 by 75 mm or 12 by 75 mm). Iodine-125

antigen reagent (0.2 ml) was added to each tube. After 0.2 ml of PCP antiserum reagent was added to each tube, the contents were mixed on a vortex-type mixer and incubated at ambient temperature for 1 h. After the incubation period, 0.5 ml of saturated ammonium sulfate solution was added to each tube; the contents were mixed on a vortex-type mixer and allowed to stand at ambient temperature for 10 min. The tubes were then centrifuged in a swinging bucket rotor at 2500*g* for 10 min. A 0.5-ml aliquot of each supernatant was transferred to standard counting vials and counted for 1 min in a gamma scintillation counter (Searle-Analytic, Inc., Des Plaines, Ill.; Model 1186; efficiency, 73%). For quantitation, calibration curves were prepared by assaying, in triplicate, standards containing 0, 12.5, 25, 50, 100, and 200 ng PCP/ml and the dose-response results were plotted on linear graph paper. The PCP concentration in each specimen was obtained by interpolation from this curve. If the specimen value was greater than 100 ng/ml, the specimen was diluted with normal human urine and the assay repeated.

Results

Performance Characteristics

Response—Adjustment of the concentration of antiserum and antigen reagents yielded a dose-response curve with an average range of 19 000 dpm for freshly prepared materials over the concentration range of 0 to 200 ng PCP/ml of urine. A typical response curve for the PCP standards is shown in Fig. 1. Examination of the lower portion of the response curve indicated that the sensitivity based on the signal detected at two standard deviation units above the background (normal control urines) was ~2 ng PCP/ml of urine.

The feasibility of using the radioimmunoassay reagents for the detection of PCP, added in vitro, in human serum was also determined. The response curve for serum standards, tested directly without any treatment, was more dynamic than that obtained for the urine standards. The background counts for the zero standards was shifted slightly upward by ~900 dpm and the counts for the 200 ng/ml standard was shifted upward by ~2700 dpm (see Fig. 1). The minimum concentration of PCP that could be reliably detected in serum was ~2 ng/ml.

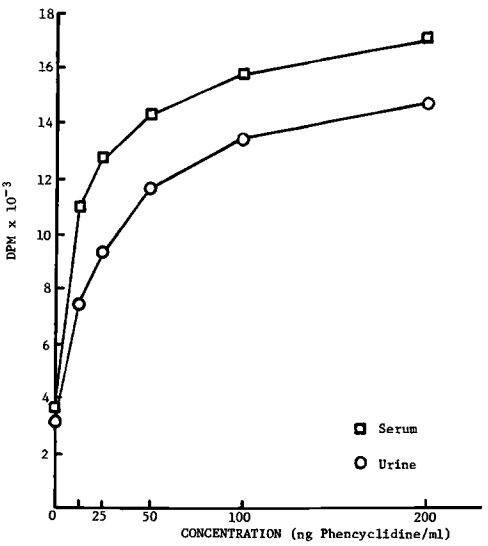

FIG. 1—*Typical PCP response curves with human serum and urine standards.*

In order to assess the maximum usable sensitivity for urine specimens, the effect of pH, specific gravity, protein, and bacterial contamination were determined in vitro. Varying the pH from 4.5 to 9.0 produced the equivalent of less than 1 ng/ml change in the negative (0 ng/ml) control. Over the range of specific gravity from 1.005 to 1.040, there was also no significant change in response for the negative control. Addition of 0 to 20 mg/ml of protein (in the form of human albumin) produced no measurable change in response. When eleven urine specimens containing bacteria (nitrite-positive, which is usually indicative of urinary tract infection) were examined, values of 9 to 10 ng/ml were obtained.

Specificity—Three chemically related compounds, two of which have been reported as urinary metabolites of PCP [*18*], were evaluated for interaction in the radioimmunoassay for PCP. Addition of either phenylcyclohexylamine, the dealkylated metabolite, or ketamine hydrochloride in the range of concentrations of 50 to 5000 and 100 to 100 000 ng/ml, respectively, to the normal urine control resulted in no significant reactivity. The mono-hydroxylated PCP metabolite, when present at 100 to 100 000 ng/ml, did react at about 10% of the equivalency of PCP.

Stability—The stability of the PCP radioimmunoassay reagents was studied as a function of time and temperature. The performance of the reagents, determined initially, at two weeks, and at monthly intervals thereafter for six months, indicated that the relative percentage of unbound ^{125}I antigen (dpm of normal human urine/dpm standard × 100) was essentially unchanged at 4°C, slightly decreased at 25 and 37°C, and significantly altered at 45°C after storage for at least three months (see Fig. 2). The decrease in response at 45°C exceeded that resulting solely from radioisotopic decay.

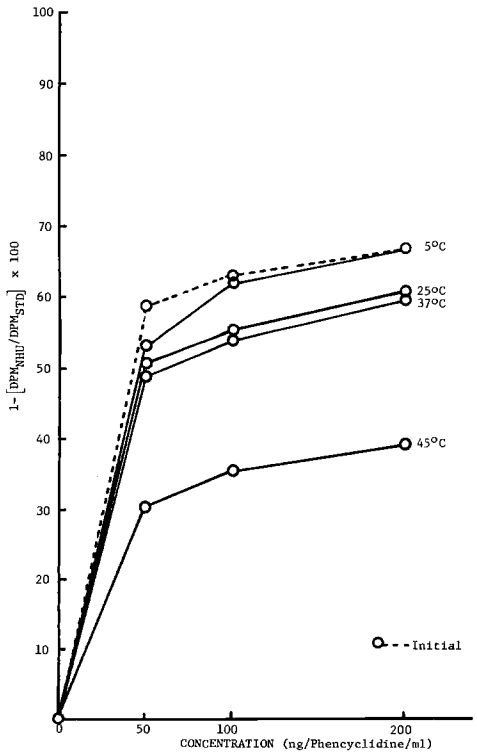

FIG. 2—*Relative percentage of unbound antigen after storage for three months at the indicated temperature.*

Precision—The precision of the radioimmunoassay was assessed by assaying on a blind basis four urine samples containing 20 to 175 ng PCP/ml. The summary of a total of 60 determinations on each of four urine specimens (five times per run, twice daily per analyst [two analysts] for three days) is listed in Table 1. Results indicated that there was no significant difference in reproducibility or repeatability either within assay or between assay.

Recovery—Recovery experiments were conducted with four urine specimens that contained known amounts of PCP. The data demonstrated that the recovery (amount found/amount added) was 93 to 109% at the 20 to 175 ng/ml concentration levels (see Table 2).

Clinical Testing

Background Values—To determine the potential for false positive results from a randomly selected population, a total of 457 urines (240 males and 217 females) were tested for PCP. The results are expressed in terms of equivalents *E* of the unlabeled PCP standard per millilitre (PCP *E*/ml) because the number and proportion of metabolites may vary with each subject. Using a cutoff value of 100 ng PCP *E*/ml, to distinguish positives from negatives, all urines were negative. Quantitatively, the majority, 89.5%, of the specimens equaled zero and a total of 94.6% contained 10 ng PCP *E*/ml or less. The highest value obtained was equivalent to 25 ng PCP/ml (see Table 2).

Thirty random human serum samples were also examined for PCP with the radioimmunoassay reagents. All of these specimens were found to contain less than 2 ng PCP *E*/ml.

Cross-Reactivity—Twenty commonly prescribed drugs were tested for cross-reactivity

TABLE 1—*Summary of reproducibility*[a] *and recovery data.*[b]

Concentration Added, ng/ml	Mean Concentration Found, ng/ml	Within Assay		Between Assay		% Recovery
		±SD[c]	±CV,[d] %	±SD	±CV, %	
20	21.9	3.8	17.4	5.1	23.3	109.5
55	51.6	7.4	14.3	8.8	17.0	93.8
120	113.9	16.3	14.3	19.5	17.1	94.9
175	163.7	22.4	13.7	17.2	10.5	93.5

[a] Within-assay and between-assay variance determined according to method of Rodbard [*20*].
[b] Concentration found/concentration added, expressed as percent.
[c] *SD* = standard deviation.
[d] *CV* = coefficient of variation.

TABLE 2—*PCP equivalents for random urine specimens.*

Range of PCP Equivalents, ng/ml	Percentage	Number
0	89.5	409
0-5	6.1	28
5-10	2.4	11
10-15	1.5	7
15-20	0.2	1
20-25	0.2	1

in urine samples obtained from subjects who had received the medication. No cross-reactivity was observed with any of the compounds listed in Table 3.

Correlation Studies—Qualitative correlation was obtained by comparison of the 135 radioimmunoassay results (for specimens obtained from suspected abusers) with independently acquired data from GC/MS, GC, and TLC. The 100 ng/ml cutoff value was used for all four methods to differentiate positive and negative PCP specimens. For 66 specimens analyzed by radioimmunoassay and GC/MS, there was 95.4% agreement. For 43 specimens analyzed by radioimmunoassay and GC, there was 93.0% agreement. Twenty-six specimens, analyzed by radioimmunoassay and TLC, were in 100% agreement (see Table 4).

Linear regression analyses were performed to obtain quantitative correlations between radioimmunoassay and either GC/MS, GC, or TLC on the values obtained. Sixteen

TABLE 3—*List of noninterfering drugs.*

Name	Amount Administered, mg
Aminopyrine (Pyramidon®)	300
Amobarbital (Amytal®)	65
Amphetamine (Benzedrine®)	5-10
Butabarbital (Butisol Sodium®)	50
Caffeine	260
Chlordiazepoxide (Librium®)	10
Chloroquine (Aralen® phosphate)	500
Chlorpromazine (Thorazine®)	10
Diphenylhydantoin (Dilantin®)	100
Glutethimide (Doriden®)	500
Methadone	...[a]
Methaqualone (Quaalude®)	300
Methyprylon (Noludar®)	300
Morphine	10mg/70 kg
Oxyphenbutazone (Tandearil®)	100
Pentobarbital (Nebutal®, sodium)	100
Phenobarbital (Luminal Ovoids®)	64
Phenylbutazone (Butazolidin®)	100
Promethazine (Phenergan®)	25
Secobarbital (Seconal®, sodium)	100
Trifluoperazine hydrochloride (Stelazine®)	1

[a] Urine contained >500 ng/ml.

TABLE 4—*Comparison of PCP results by radioimmunoassay with those by GC/MS, GC, and TLC.*

	Radioimmunoassay Analysis	
Other Analyses	Positive	Negative
GC/MS positive	22 (33.3%)[a]	1 (1.5%)
GC/MS negative	2 (3.0%)	41 (62.1%)
GC positive	15 (34.9%)	2 (4.7%)
GC negative	1 (2.3%)	25 (58.1%)
TLC positive	26 (100.0%)	0
TLC negative	0	0

[a] Number of specimens giving indicated result/total number of specimens tested, expressed as a percentage.

clinical specimens, unequally distributed in concentration from 0 to 15 μg/ml, were independently analyzed by GC/MS. The values for slope, y intercept, and correlation coefficient were 0.66, 33.3, and 0.997, respectively. Additional clinical specimens, equally distributed over the concentration range of 0 to 1000 ng PCP/ml by radioimmunoassay, were also analyzed by GC/MS. The values for the y intercept, slope, and correlation coefficient were 42.5, 1.07, and 0.97, respectively. Results of linear regression analysis, using different specimens in each study, showed the correlation coefficient values listed in Table 5 when the results from radioimmunoassay were compared with those from GC and TLC.

Discussion

Although reagent sensitivity could be increased to detect less than 2 ng/ml by decreasing the amount of antiserum and antigen in the formulation, it is doubtful whether greater sensitivity would be useful under clinical conditions. The range of background values with both random specimens and with specifically controlled variables (pH, specific gravity, protein, and nitrite-producing bacteria) precludes more than 90% confidence for any result below 2 ng/ml of urine.

The lack of cross-reaction of the radioimmunoassay reagents with phenylcyclohexylamine, even at 5000 times the concentration of PCP, is both a desirable and a necessary characteristic. If antiserum specificity for the three-ring structure were not maintained, interaction could occur with a variety of bicyclic phenylcyclohexylamine analogs.

TABLE 5—*Linear regression analysis of results by radioimmunoassay (RIA) and GC/MS, GC, or TLC.* [a]

Parameter	GC/MS [b]	RIA [b]	GC [b]	RIA [b]	TLC [c]	RIA [c]
Phencyclidine concentration	0	0	26	2	0.5	1.29
	27	25	40	17	0.5	1.38
	25	49	50	31	0.5	1.60
	94	109	200	70	2.6	1.67
	109	166	300	129	0.8	2.18
	149	237	260	147	1.2	2.70
	300	295	320	172	2.7	2.98
	280	364	260	187	2.0	3.74
	322	406	390	201	2.3	4.74
	425	488	210	239	3.9	5.02
	565	575	600	241	2.5	5.18
	395	680	510	290	2.5	8.13
	765	850	320	293	12.6	14.8
	720	912	340	298	9.3	18.4
	1040	1065	430	538		
			730	949		
			1090	1086		
			1460	1343		
			2180	2032		
y intercept	42.5		−61.8		1.06	
Slope	1.07		0.97		1.35	
Correlation coefficient, r_{xy}	0.97		0.97		0.91	

[a] For linear regression analysis, y was the value obtained by RIA and x was the value obtained by the alternate method.

[b] ng/ml.

[c] μg/ml.

The unavailability of the other possible mono-hydroxylated metabolite, 1-(3-hydroxy-1-phenylcyclohexyl)piperidine, did not permit determination of its extent of cross-reactivity. Qualitative correlation at the 100 ng/ml limit by radioimmunoassay was highest with TLC (see Table 4). This was due primarily to the specimen concentrations, which in all cases were significantly greater than 300 ng PCP *E*/ml, whereas some of the specimens for the GC/MS testing were near the 100 ng/ml limit value. The comparison demonstrated the adequacy of using the 100 ng/ml limit value for abuse screening. Use of a lower limit value, 75 or 50 ng/ml, would be expected to decrease the confirmatiom rate of abusers relative to GC and TLC.

The quantitative correlation studies demonstrated excellent agreement with three other methods currently in use. The two separate GC/MS correlation studies resulted in equivalent intercept and correlation (coefficient) values. Considering that the GC/MS determinations were made on different specimens in different laboratories, the results were quite startling. The positive intercept value (32 to 41 ng/ml), showing a slight positive bias, may be due in part to the need for extraction of the specimens in GC/MS and in part to detection of only the PCP *m/e* peak in GC/MS. Conversely, specimens were tested without extraction by radioimmunoassay and both PCP and the mono-hydroxylated metabolite were detected. These comparison studies also indicated the feasibility of routinely performing PCP determinations at trace levels by radioimmunoassay.

It is hoped that in near future these PCP radioimmunoassay reagents can be applied to those clinical and toxicological situations where quantitative determination of PCP at levels well below 100 ng/ml is essential.

In conclusion, the radioimmunoassay for PCP is sensitive, specific, and rapid. It is well suited for small- or large-scale testing.

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